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Astrovirology in marine systems: how virus-microbe interactions accelerate evolution and generate organismal diversity

Hong-Yan Shih & Nigel Goldenfeld

Department of Physics &

Carl R. Woese Institute for Genomic Biology
University of Illinois at Urbana-Champaign, USA

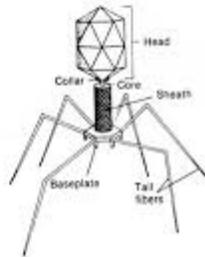
Institute of Physics, Academia Sinica, Taiwan

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A central question from NASA for biology

NASA Astrobiology Roadmap Objective 5.2—Co-evolution of microbial communities. Examine the metabolic and genetic interactions in microbial communities, including viruses, which have determined major geochemical processes and changes on Earth. Investigate how these interactions shape the evolution and maintenance of metabolic diversity in microbial communities. Investigate how novel microbial species establish and adapt into existing communities.



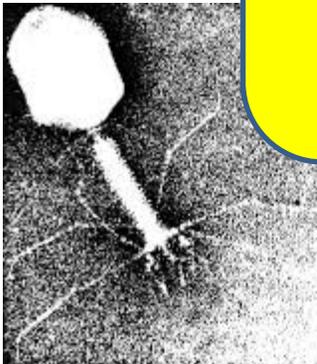
Predator

Prey

A central question from NASA for biology

NASA Astrobiology Roadmap Objective 5.2—Co-evolution of microbial communities. Examine the metabolic and genetic interactions in microbial communities, including viruses, which have determined major geochemical processes and changes on Earth. Investigate how these interactions shape the diversity in microbial communities as they establish and adapt into existing communities.

But what about the environment?



Predator



Prey

Stability of microbial communities

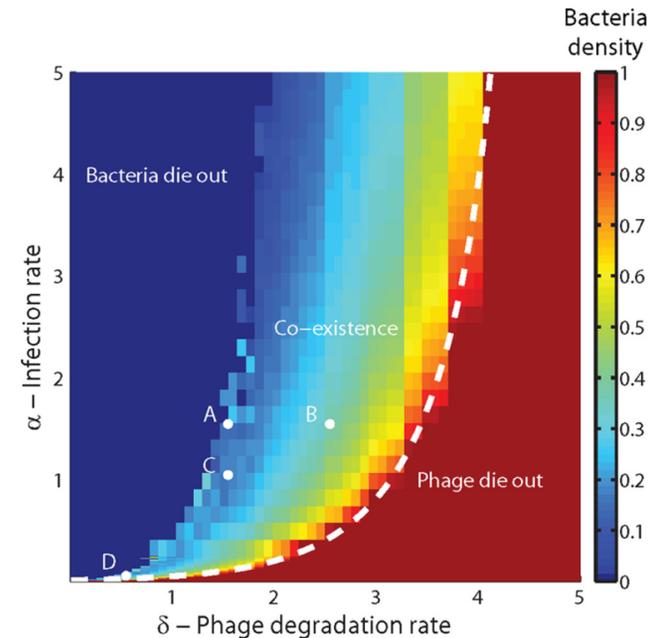
- **Problem: stability of highly diverse microbe-phage communities is difficult because of exponential growth of phage due to large burst size.**
- How are such communities stabilized, and what controls their diversity?
- Spatial variability promotes coexistence but still difficult to achieve. Moderate virulence of phage, a non-selfish strategy in long-term, also promotes stability and coexistence

Astrobiology Roadmap Objective 5.2—Co-evolution of microbial communities. Examine the metabolic and genetic interactions in microbial communities, including viruses, which have determined major geochemical processes and changes on Earth. Investigate how these interactions shape the evolution and maintenance of metabolic diversity in microbial communities. Investigate how novel microbial species establish and adapt into existing communities. Marais et al. (2008)

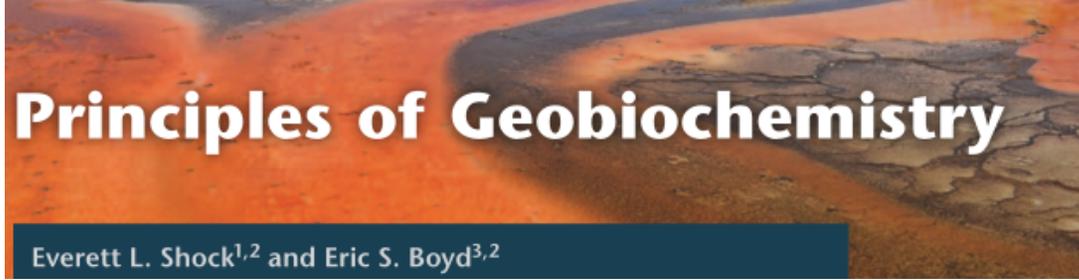
Happel and Stadler (1998)

Rosvall et al, PRE (2006); Heilman et al. (2010)

Weitz , *Quantitative Viral Ecology* (2016)



What is life?



Principles of Geobiochemistry

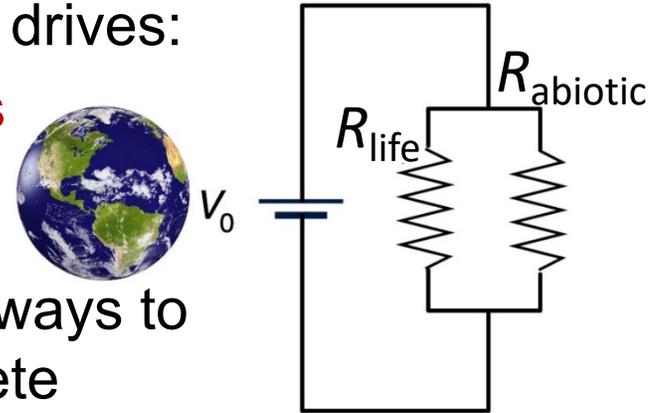
Everett L. Shock^{1,2} and Eric S. Boyd^{3,2}

The basic premise of geobiochemistry is that life emerged on Earth where there were opportunities for catalysis to expedite the release of chemical energy in water–rock–organic systems. In this framework, life is a planetary response to the dilemma that cooling decreases the rates of abiotic processes to the point that chemical energy becomes trapped.

Catalysis via metabolism releases the trapped energy, and life benefits by capturing some of the energy released. Out of necessity, biochemical processes have geochemical origins, and geobiochemistry asserts that these origins can be revealed by mapping reaction mechanisms onto deep time. We propose five principles that should help guide research in the emerging field of geobiochemistry.

Living systems utilize multi-scale flows

- Environment provides energy input (light, nutrient and chemical gradient etc) that drives:
 - Abiotic, physico-chemical processes
 - Living systems
- Living systems use novel catalytic pathways to accelerate reaction rates and outcompete abiotic processes
- Living systems discover these pathways through the **flow of information**
 - Evolutionary mechanisms impact ecology and global planetary environment
 - Horizontal gene transfer as a powerful evolutionary mechanism



Living systems utilize multi-scale flows

- Environment provides energy input (light, nutrient and chemical gradient etc) that drives:

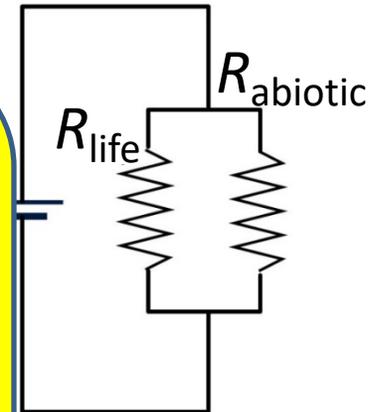
- Abiotic
- Living

- Living systems accelerate abiotic processes
- Living systems thrive through time

- Evolutionary mechanisms impact ecology and global planetary environment
- Horizontal gene transfer as a powerful evolutionary mechanism

This talk:

Tiny marine cyanobacteria (*Prochlorococcus spp.*) and their phage as an example of how life uses information flow to thrive and occupy new planetary niches



Ecosystem as a non-equilibrium steady state from multi-scale feedback

- **Thermodynamics**: environment provides energy input (light, nutrient and chemical gradient) that drives metabolic processes and adds selection to ecosystems
- **Ecology** has two aspects, currently not well-unified:
 - (1) **Population dynamics**: reflecting selection through birth/death of organisms that are coupled by multi-scale spatiotemporal interactions.
 - (2) **Metabolism**: metabolic network inside individuals/ organisms as a global electron market that transduces energy flows.
- **Information flow**: living systems outcompete abiotic processes by using information (via mutation, horizontal gene transfer etc) to find pathways.

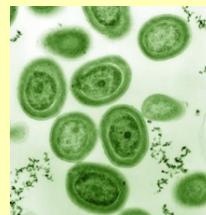
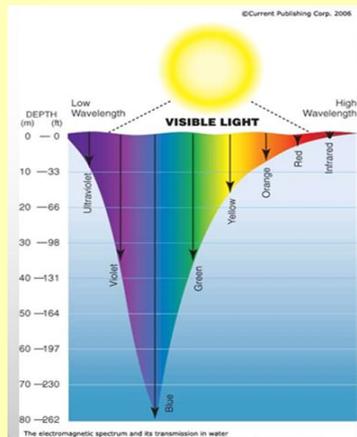
Ecosystem as a non-equilibrium steady state from multi-scale feedback

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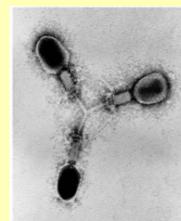
Collective evolution

How life uses information to occupy niches
in marine microbe-virus ecosystem

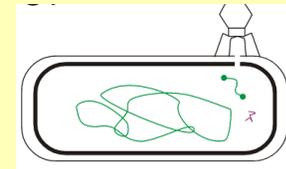
photon gradient + population dynamics + information flow



Cyanobacteria

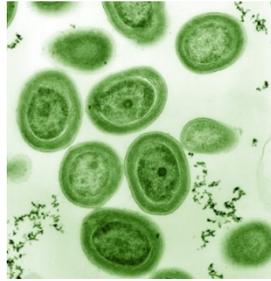


Cyanophage

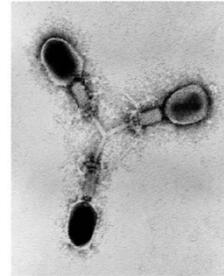


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Outline: Ecosystem stability through collective effects



Cyanobacteria



Cyanophage

- Horizontal gene transfer as a driving force in evolution and ecology
- Modeling the coevolution and collective dynamics of cyanobacteria and cyanophage
- Explain the range expansion and niche stratification of cyanobacteria
- Predation stabilizes the ecosystem

Role of collective effects

- Usual assumption:
 - Bacteria = prey
 - Phage = predator
 - Model by some appropriate Lotka-Volterra dynamics
- Relationship between bacteria and phage is much more complex:
 - **Coevolutionary arms race** between bacteria cell receptors and phage tail sequences → diversity of bacterial genome sequences and phenotypes
 - **Horizontal gene transfer** between bacteria mediated by phage → genetic diversity
- **Our main question:** how does the complexity of interactions between bacteria and phage influence the evolution, dynamics and stability of communities?
 - How can we account for the diversity and growth of the pan-genome of bacteria and phage?
- **Our approach:** look at rich metagenomics datasets of one of the most abundant organisms on the planet, *Prochlorococcus spp.* and its phage.

Cyanobacteria and cyanophages

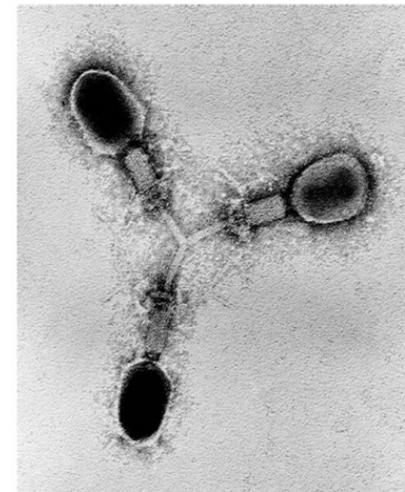
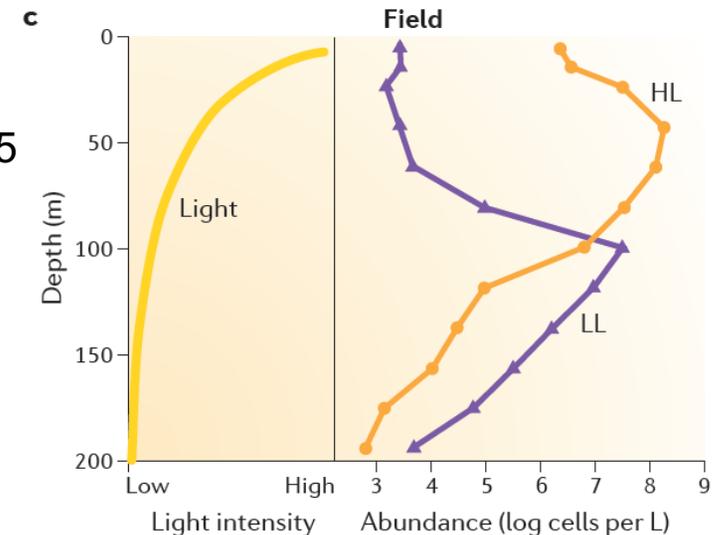
Prochlorococcus:

- most abundant photosynthetic organism (10^{27} cells)
- **highly streamlined genome** (1700 genes in 1.65 Mbp)
- dilute in the ocean ($10^6/L$)
- fix same amount of carbon as global croplands
- **no bacterial immune system (CRISPR or prophages)**
- genetic diversity mainly arises from genes acquired by HGT that code for cell surface molecules to resist phage attack
- **Huge pan-genome** (84,000 genes)
- **niche stratification** (Low- and high-light adapted ecotypes)

Cyanophages:

- lytic double-stranded DNA tailed phages in T4, T7 and lambdoid groups
- **carry photosynthesis genes**

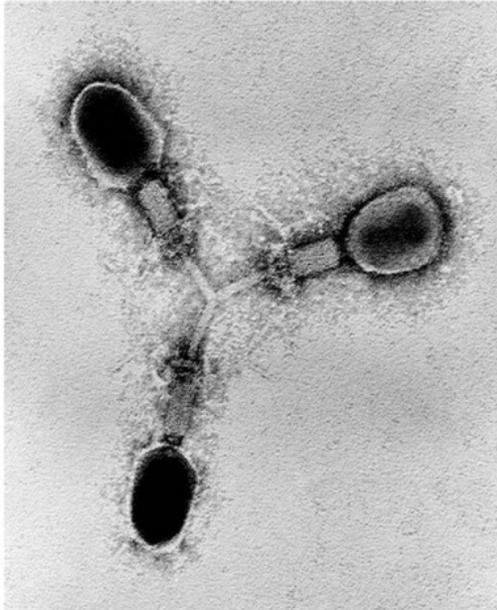
Lindell, *Nature* (2005)



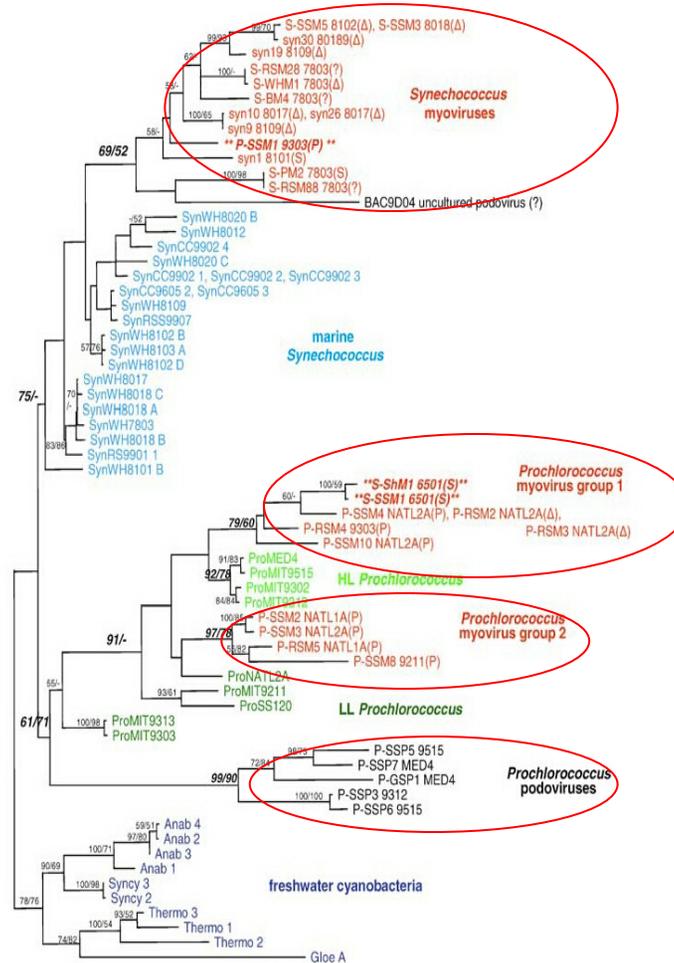
Biller et al. *Nature Rev. Microb.* 13(1), 13 (2015)

Gene transfer between host and virus

Sullivan et al. , PlosBiol (2006)



Phage with photosynthesis genes have higher burst size during lysis.



PsbA gene acquired by phage

Phylogeny of psbA gene in cultured cyanobacteria and cyanophages

Is there a benefit to microbes of viruses?

“Therefore, mounting evidence indicates that **host-like genes acquired by phages undergo a period of diversification in phage genomes** and serve as **a genetic reservoir for their hosts**. Thus, a complex picture of overlapping phage and host gene pools emerges, where **genetic exchange across these pools leads to evolutionary change for host and phage**. Fully understanding the mechanisms of microbial and phage coevolution clearly requires an improvement in our ability to **quantify horizontal gene transfer** at the whole and partial gene level and in our ability to accurately estimate the relative fluxes into and out of these pools.” (Sullivan et al. 2006)

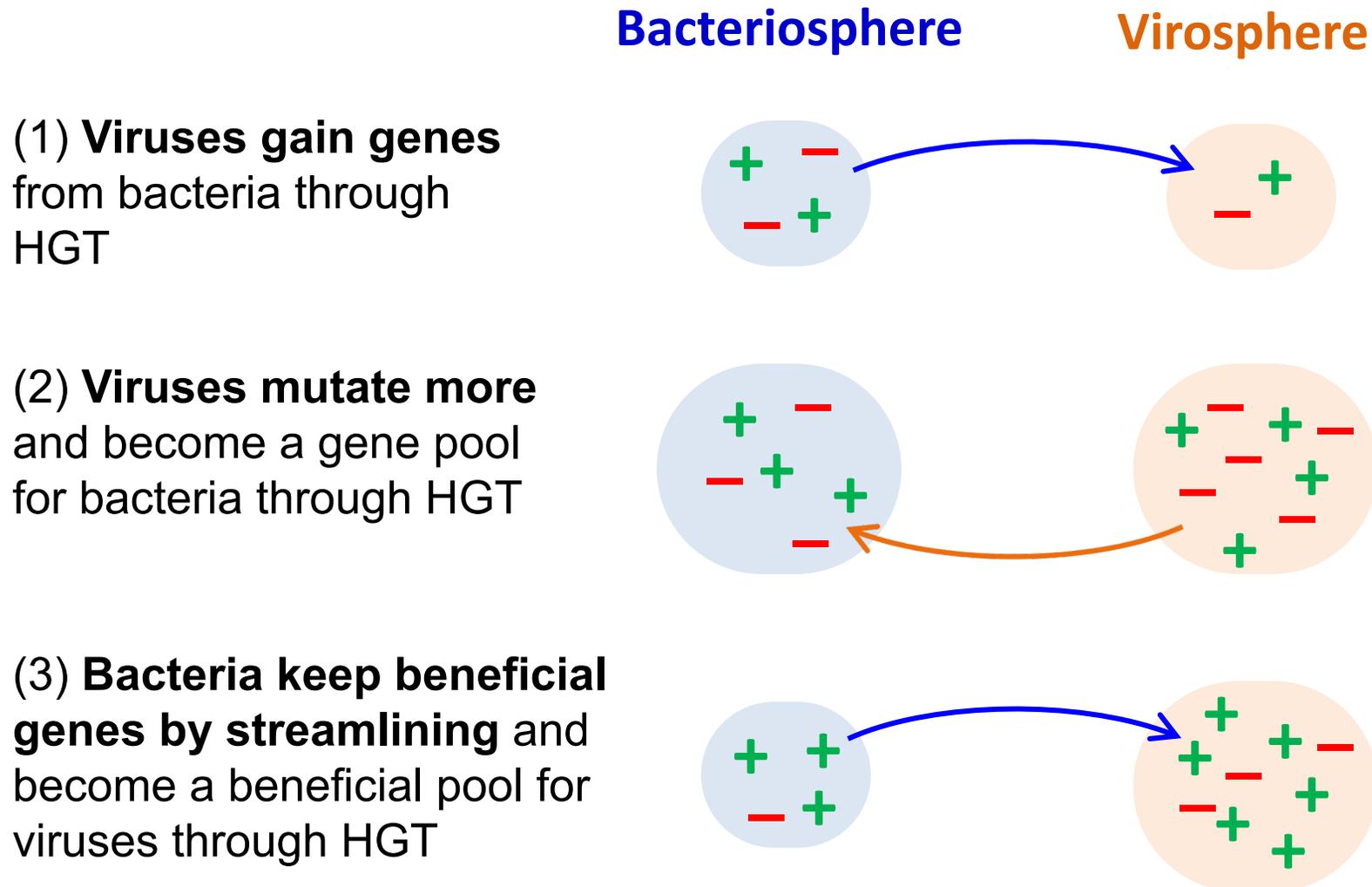
Yes: microbe-phage interactions create a global reservoir of photosynthetic genes, benefiting both microbes and phages.

(E. Anderson (1966), N. Anderson (1970), S. Sonea (1988, 2001), M. Syvanen (1984) & many others, including L. Villareal, Weinbauer, Ochman, Lawrence, Groisman, Hatfull, Hendrix, Brussow ...)

Minimal stochastic model of interaction between bacteria and virus

Goal: can a collective, mutually
beneficial state between microbes and
virus emerge through antagonistic
interactions?

HGT and genome streamlining create a reservoir of beneficial genes

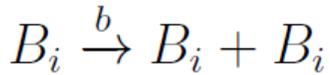


Collective effects prevent extinction

- Sequence of stochastic models with different interactions and assumptions to understand qualitative features of phase diagram
 1. Predation and evolution of barrier to predation (g_{BP})
 2. HGT dynamics: generalized transduction
 3. Phenotypic effect of photosynthesis genes on both bacteria and phage (g_{PS})
 4. Consequences of small genome size: Muller's ratchet
 - Highly streamlined cyanobacterial genome
 - Small capacity for phage payload
 5. Cyanobacteria & phage in a photon gradient

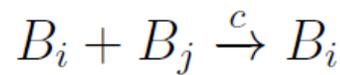
Model 1: Coevolution of barrier to predation stabilizes bacteria-phage community (1)

Birth



Bacteria with allele g_{BP}^i

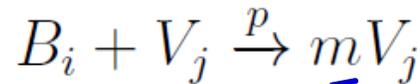
Competition



Death



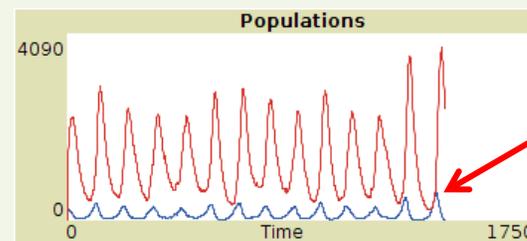
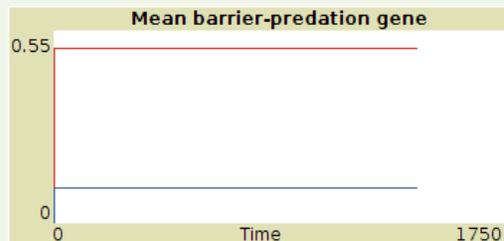
Predation



Phage burst size

- Predator-prey model (Lotka-Volterra) with negligible mutation rate is unstable under **high predation pressure**.

(red=phage, blue=bacteria)



Populations collapse

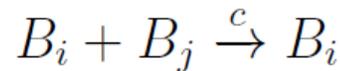
Model 1: Coevolution of barrier to predation stabilizes bacteria-phage community (2)

Birth



Bacteria with allele g_{BP}^i

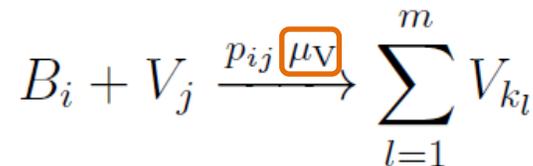
Competition



Death

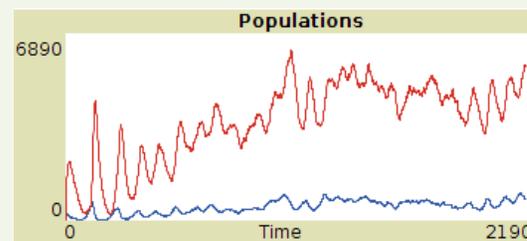
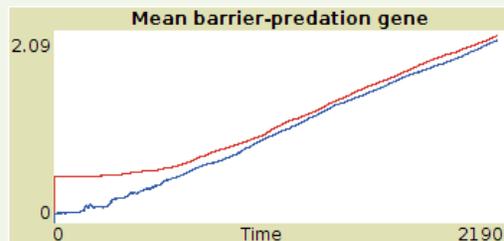


Predation



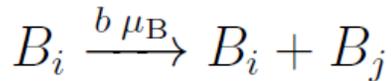
V_{kl} : daughter phages with genes mutated from mother

→ **Mutation** ensures a persistent coexisting state by continually providing more competitive genes to bacteria and phage separately (red=phage, blue=bacteria)



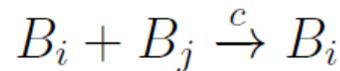
Model 2: HGT of barrier to predation stabilizes bacteria-phage community (1)

Birth



Bacteria with allele g_{BP}

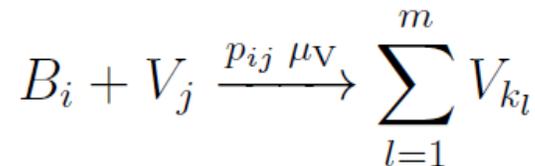
Competition



Death

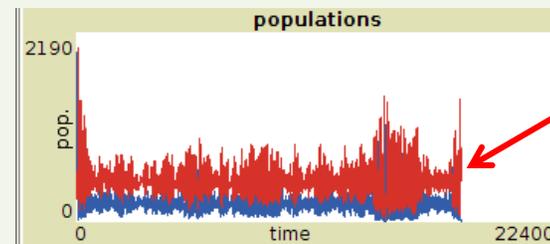
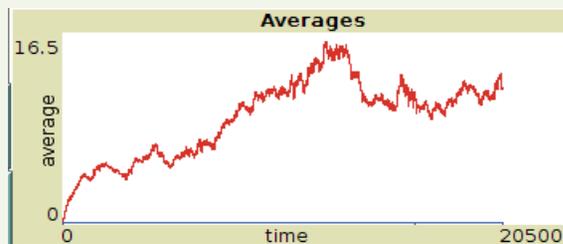


Predation



- Predator-prey model is **unstable** under **unequal mutation rates** ($\mu_V \gg \mu_B$).

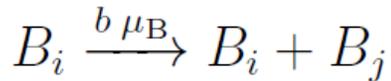
(red=phage, blue=bacteria)



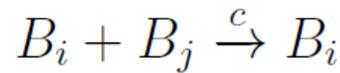
Populations collapse

Model 2: HGT of barrier to predation stabilizes bacteria-phage community (2)

Birth



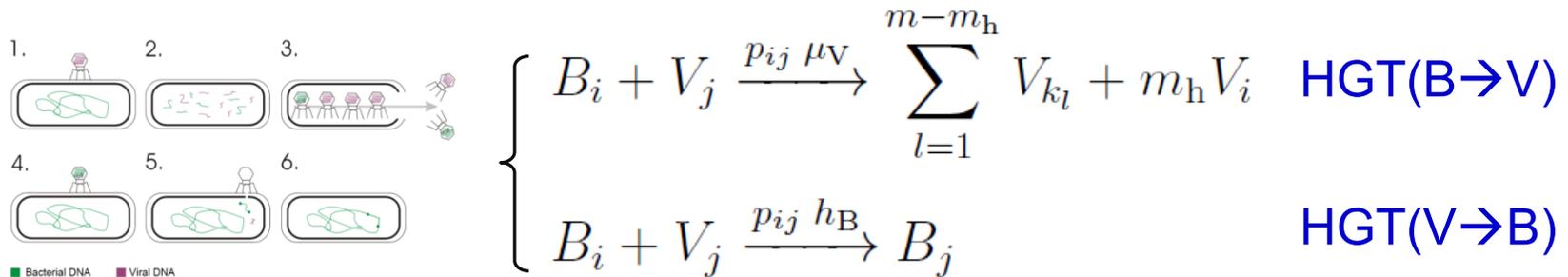
Competition



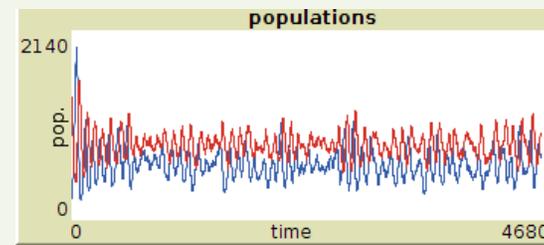
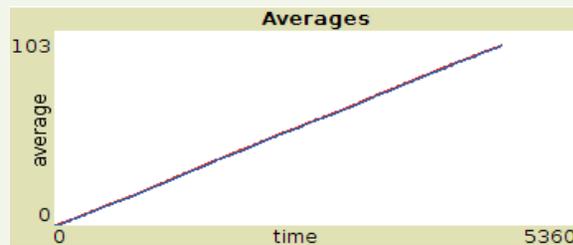
Death



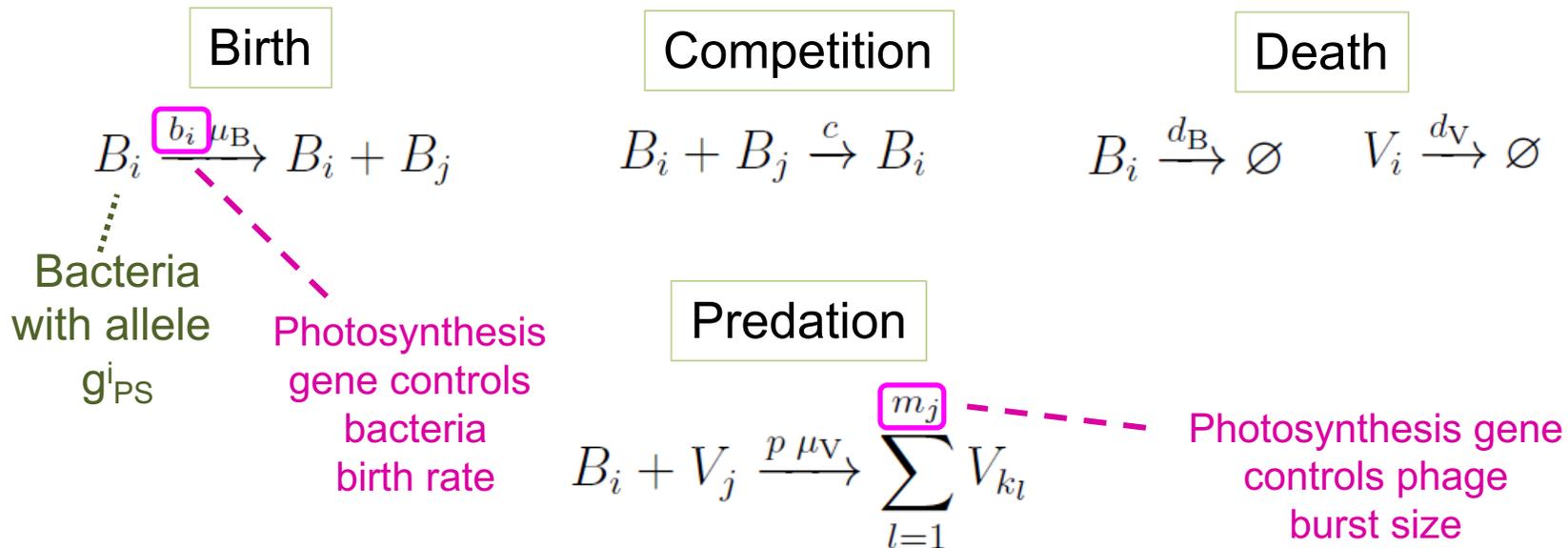
Generalized transduction



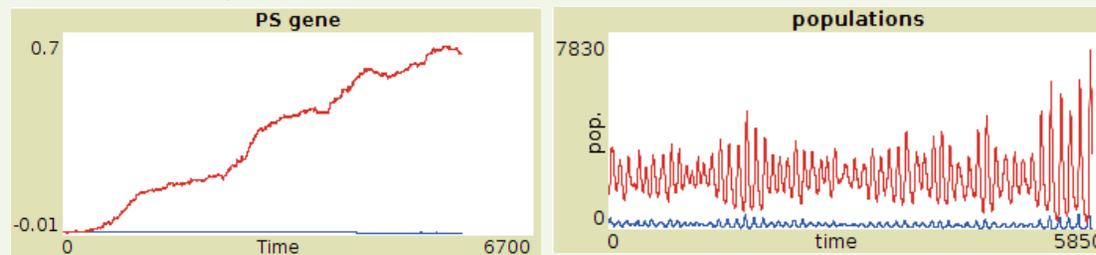
→ **Horizontal gene transfer** ensures a persistent coexisting state by continually replenishing competitive genes **between bacteria and phage**.
(red=phage, blue=bacteria)



Model 3: HGT of photosynthesis gene stabilizes bacteria-phage community (1)

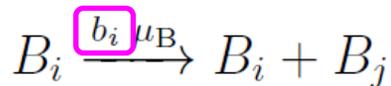


- Predator-prey model is **unstable** under **unequal mutation rates** ($\mu_V \gg \mu_B$).
(red=phage, blue=bacteria)

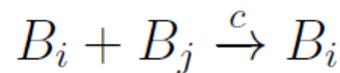


Model 3: HGT of photosynthesis gene stabilizes bacteria-phage community (2)

Birth



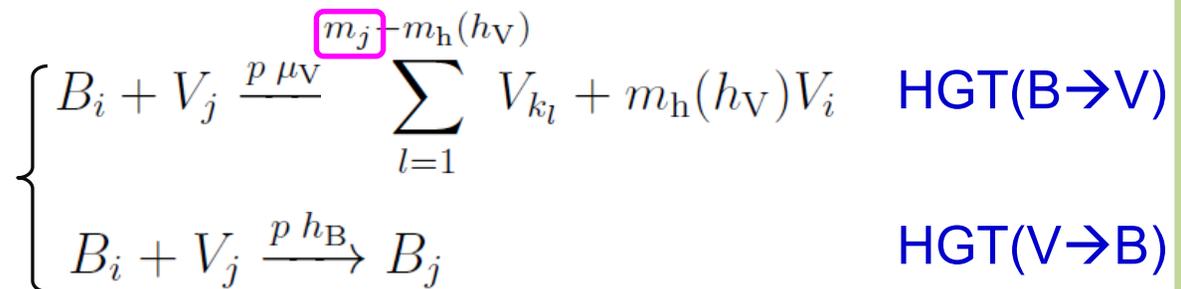
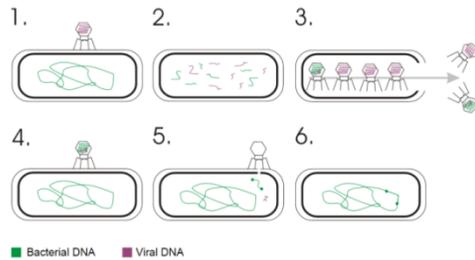
Competition



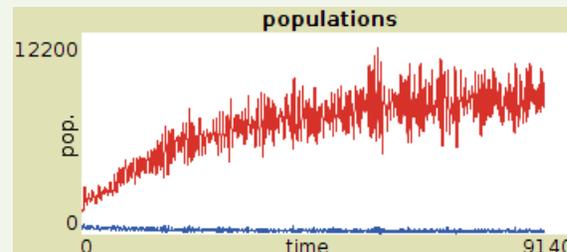
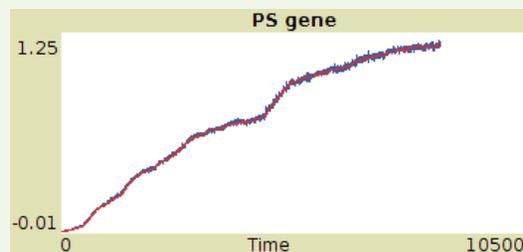
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Generalized transduction

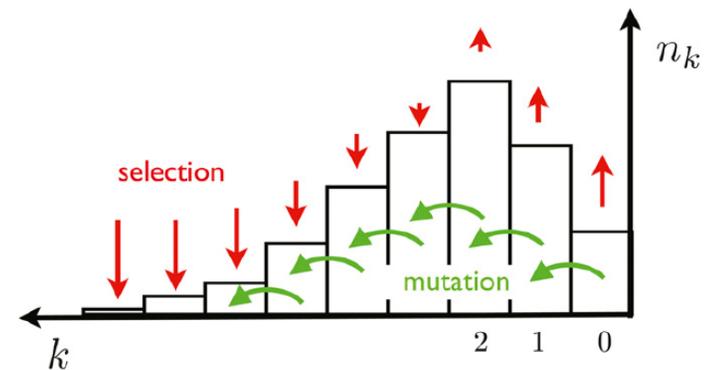
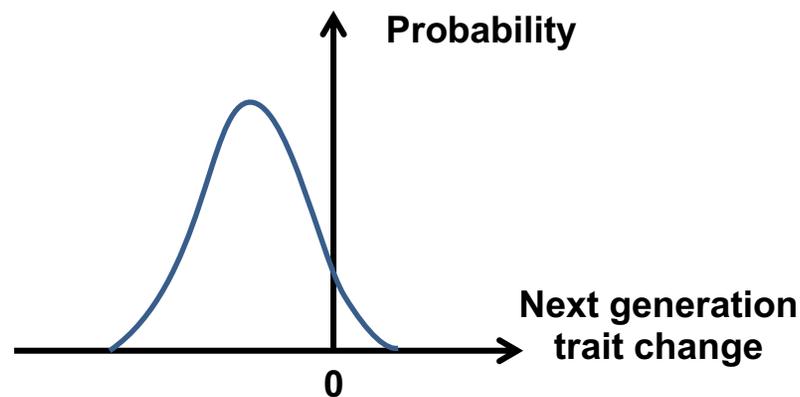


→ **Horizontal gene transfer** ensures a persistent coexisting state by continually replenishing competitive photosynthesis genes **between** bacteria and phage



Missing: deleterious mutations

- For well-adapted species, most mutations are neutral or deleterious.
- Accumulating deleterious gene from mutation will eventually lead small population to extinction (**Muller's ratchet**).

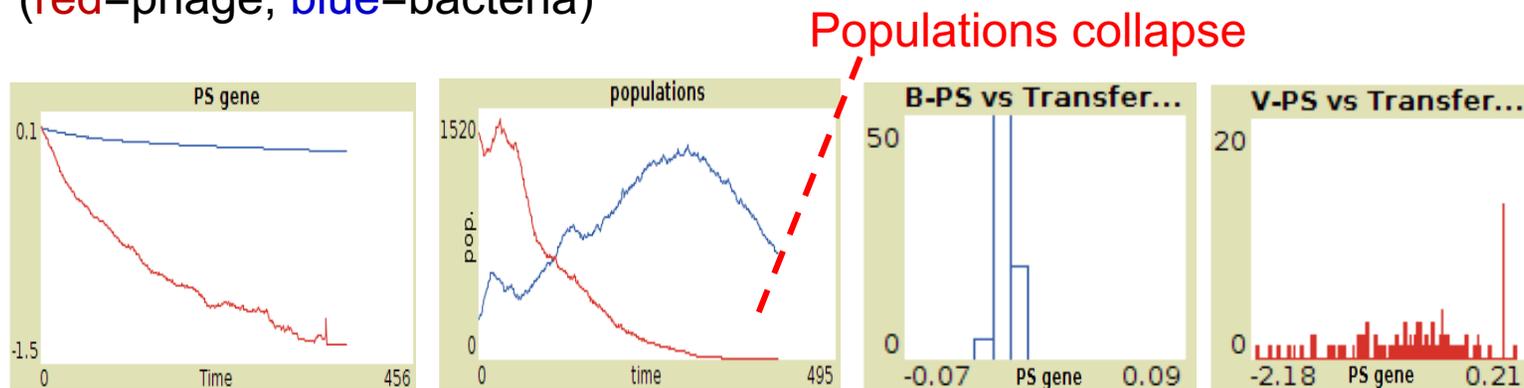


Goyal et al. *Genetics* 191 (4), 1309 (2012)

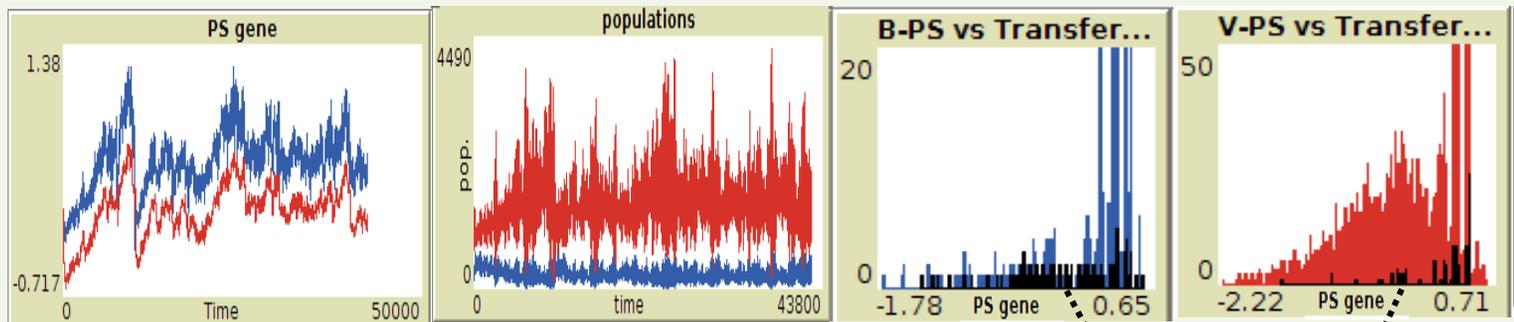
- As population decreases, **demographic fluctuations** become larger, leading population to extinction faster (**mutational meltdown**).
- In **range expansion**, demographic fluctuations are significantly large at the front.

Model 4: Deleterious mutations of photosynthesis genes

- Deleterious mutations drive population to extinction: (red=phage, blue=bacteria)



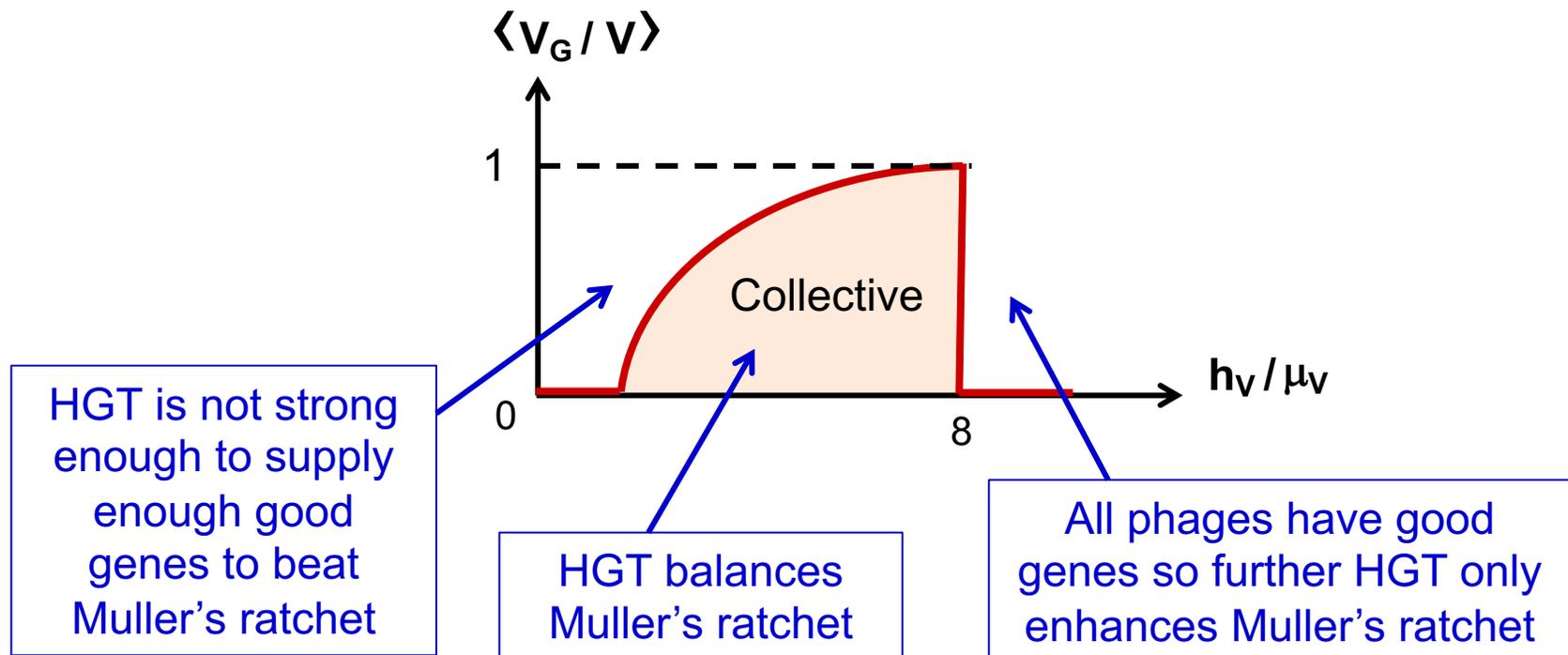
→ **Horizontal gene transfer** prevents Muller's ratchet by providing a flux of beneficial photosynthesis genes.



Transferred genes

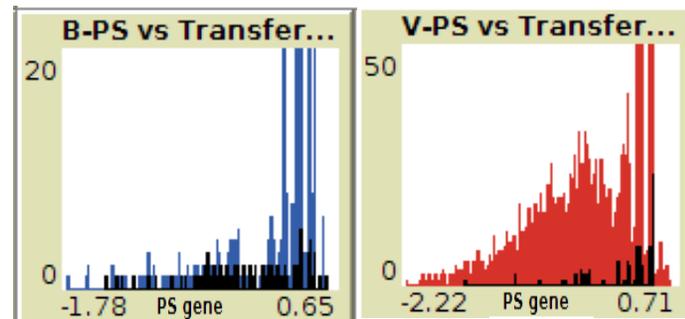
Model 4: Deleterious mutations of photosynthesis genes

- Phase diagram:
 - Fraction of viruses with beneficial photosynthesis gene ($\langle V_G / V \rangle$) as a function of horizontal gene transfer rate normalized by viral mutation rate

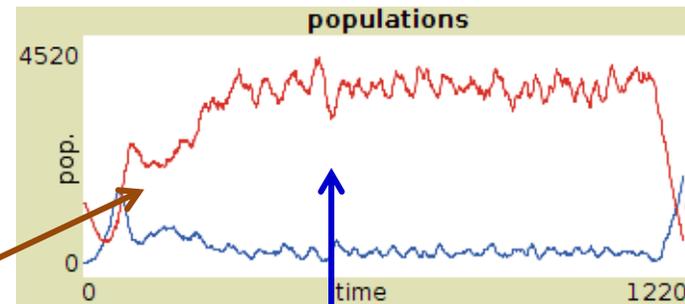


Why is this a collective state?

- Horizontal transferred genes (in black) are the main contribution to the best photosynthesis genes.



- HGT in both directions is required to maintain state



Ecological feedback

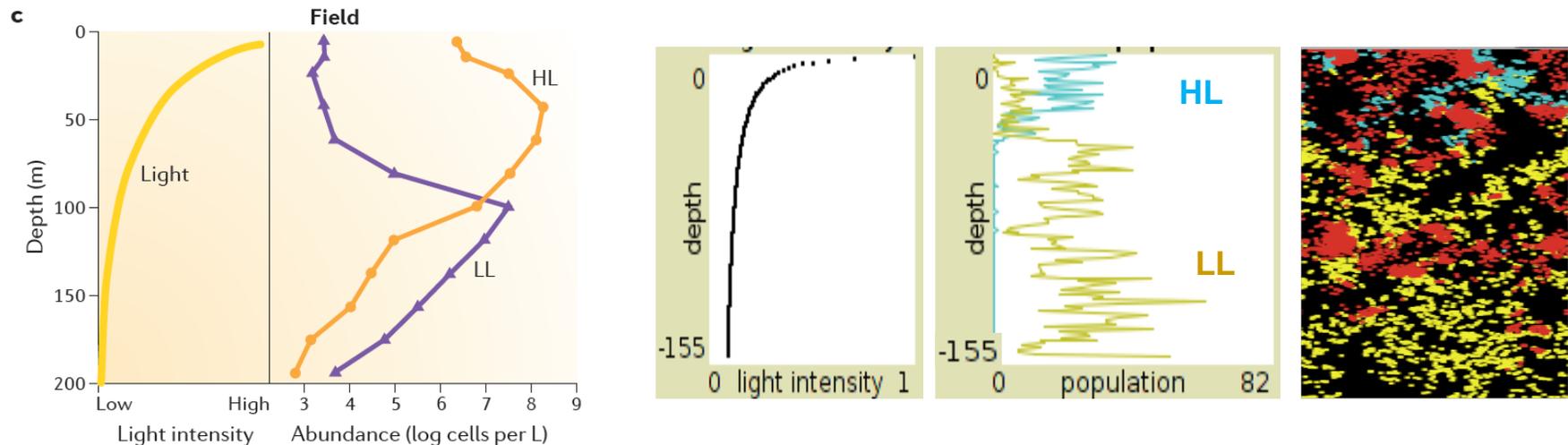
When both h_V and h_B are comparable and non-zero, collective state is stable, and burst-size grows with time.

Burst-size saturates; constant flux of beneficial PS genes from HGT, balances Muller's ratchet

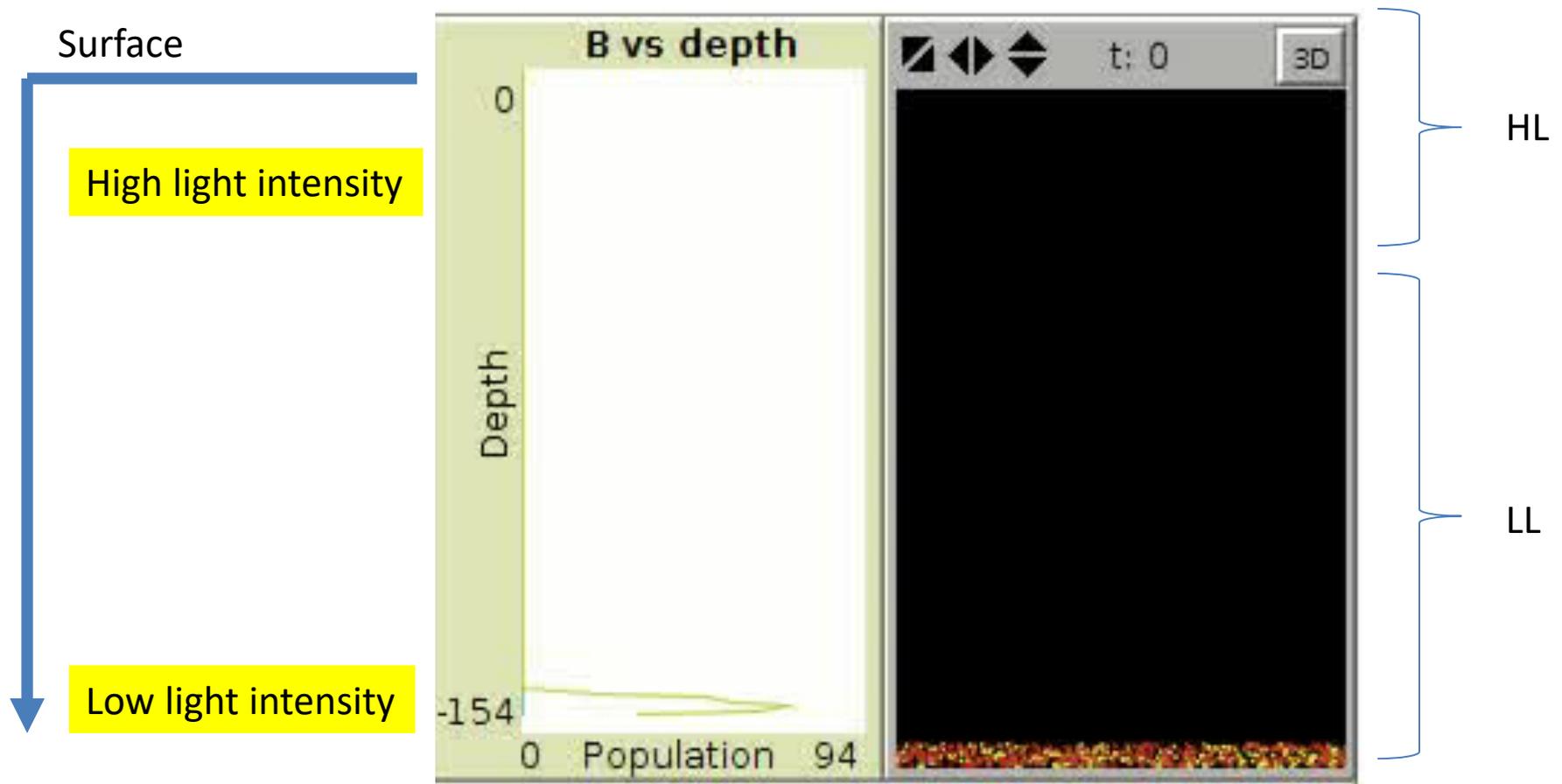
When h_B or h_V is turned off, Muller's ratchet returns and population goes extinct

Model 5: Emergence of niche stratification

- Photon density varies with depth
- Bacteria birth rate is a function of photon density and photosynthesis gene value
- Starting with low-light adapted (LL) bacteria at the bottom with phages
- Quickly mutating phages transfer photosynthesis gene to bacteria, which speeds up the formation of high-light adapted (HL) bacteria → range expansion



Emergence of niche stratification



Cyanobacteria and phage help each other!

- **Collective behavior despite predation:**
 - Bacteria have slow mutation rate compared to that of phage. Through HGT they can benefit from the phage high mutation rate, at the cost of predation.
 - Phage have fast mutation rate compared to bacteria, and so will deteriorate by Muller's ratchet. They can benefit from the bacteria low mutation rate, and acquire evolved photosynthesis genes that prevent degradation of phage fitness
- **These collective phenomena create a huge pan-genome, leading to range expansion and niche stratification**

What does the minimal model explain?

- **Prochlorococcus**

- Highly streamlined genome (1700 genes in 1.65 Mbp)

- Limited metabolic redundancy so the only avenue is to improve efficiency

- No CRISPR or prophages

- Bacteria balance risk of predation with benefit of HGT

- Huge pan-genome (84,000 genes)

- Genomic islands reflect weak bacterial defense against predation

- Niche stratification (Low- and high-light adapted ecotypes)

- Emerges from viral-mediated utilization of photon gradient

- **Cyanophage**

- Carry photosynthesis genes

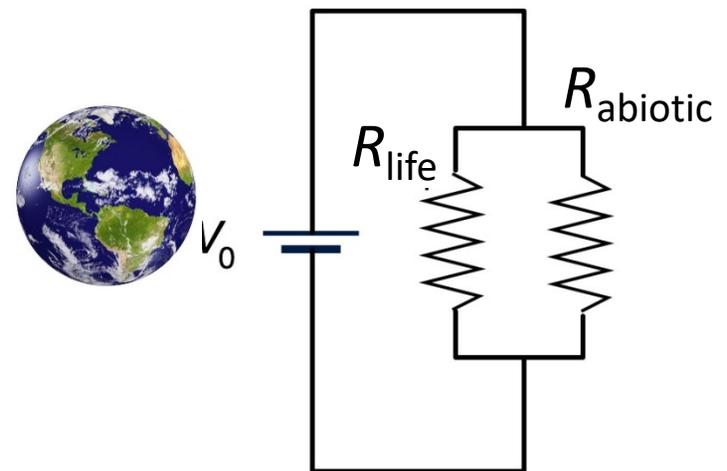
- Virus benefits from improved photosynthesis genes through larger burst size

Summary

1. Prochlorococcus (cyanobacteria) and cyanophages form a collective state through horizontal gene transfer.
2. Even though viruses are predators, they create a rapidly evolving reservoir of photosynthesis genes for their victims.
3. Even though bacteria are prey, they create a slowly evolving, stable repository of beneficial photosynthesis genes for their predators, preventing Muller's ratchet.
4. Microbe-phage interactions create a huge global reservoir of photosynthetic genes, benefiting both microbes and phages.
5. These collective effects lead to range expansion and niche stratification of the bacteria.

Take home message

- We have given an example showing how an ecosystem can evolve to occupy niches where it can utilize the energy source provided by the photon gradient.
- This happens due to information flow mediated by horizontal gene transfer.



Acknowledgements

Collaborator: Hong-Yan Shih

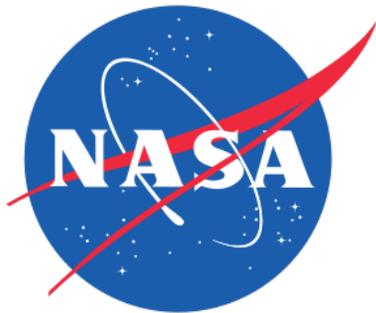
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Edelheit Family Fellowship in Biological Physics



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